

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
1 July 2004 (01.07.2004)

PCT

(10) International Publication Number
WO 2004/055155 A3

(51) International Patent Classification⁷: C12N 5/00, 5/02

(21) International Application Number:
PCT/IL2003/001030

(22) International Filing Date: 7 December 2003 (07.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/433,619 16 December 2002 (16.12.2002) US

(71) Applicant (for all designated States except US): **TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.** [IL/IL]; Senate House, Technion City, 32 000 Haifa (IL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **AMIT, Michal** [IL/IL]; 261 Yuvalim, 20 142 Misgav (IL). **IT-SKOVITZ-ELDOR, Joseph** [IL/IL]; 42 SheErit HaPleta Street, 34 987 Haifa (IL).

(74) Agent: **G. E. EHRLICH (1995) LTD.**; 11 Menachem Begin Street, 52 521 Ramat Gan (IL).

(81) Designated States (national): AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ,

CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, EG, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK (utility model), SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
10 September 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF PREPARING FEEDER CELLS-FREE, XENO-FREE HUMAN EMBRYONIC STEM CELLS AND STEM CELL CULTURES PREPARED USING SAME

(57) Abstract: The present invention is of methods of establishing and propagating human embryonic stem cell lines using feeder cells-free, xeno-free culture systems and stem cells which are capable of being maintained in an undifferentiated, pluripotent and proliferative state in culture which is free of xeno contaminants and feeder cells.

WO 2004/055155 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL03/01030

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 5/00, 5/02

US CL : 435/325, 405

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/325, 405

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
60/433,619

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, Medline, PALM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GOLDSBOROUGH ET AL. Serum-free culture of murine embryonic stem cells Focus, 1998 Vol 20, No. 1, pages 9-12, entire reference for culture conditions for mice.	1-152
Y, E	AMIT ET AL. Feeder layer- and serum-free culutre of human embryonic stem cells, Biol. of Reprod, 2004, Vol. 70, pages 837-845, entire reference for specifics demonstrated to work for human and mouse ES cells.	1-152
Y, P	AMIT ET AL. Human feeder layers for human embryonic stem cells, Biol. of Reprod, 2003, Vol. 68, pages 2150-2156, entire reference.	1-152
Y, P	PEI ET AL. Serum free culture of rhesus monkey embryonic stem cells, Arch. Androl., 2003, Vol. 49, pages 331-342, entire reference for similarity of culture conditions among other primates.	1-152
Y	MURDOCH ET AL. Human embyronic derived hematopoietic repopulating cells require distinct factors to sustain in vivo repoplatng function, Exp. Hematol, 2002, Vol 30, pages 598-605, for culture conditions of pluripotent cells.	1-152

☒ Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

29 April 2004 (29.04.2004)

Date of mailing of the international search report

28 JUL 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Joseph T. Weitach

Telephone No. (571)272-1600

Janice Ford
for

INTERNATIONAL SEARCH REPORT

PCT/IL03/01030

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AMIT ET AL. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture, Dev. Biol. 2000, Vol 227, pages 271-278, entire references for conditions required by human embryonic stem cells.	1-152